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ESTROGEN REGULATION OF PTHrP GENE EXPRESSION IN THE RAT NERVOUS SYSTEM IS TISSUE-SPECIFIC.

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Though the parathyroid hormone-related protein (PTHrP) gene is exquisitely regulated by estrogen in the rat uterus, the tissue-specificity of this response has not yet been studied. Another estrogen target, the brain expresses PTHrP in discrete regions including the cerebral and cerebellar cortex, and hippocampus (P.N.A.S. 87:108, 1990) suggesting multiple roles for PTHrP in the CNS. In this study, we examined the expression of PTHrP in the hypothalamus, pituitary gland and sciatic nerve of sham-operated and 28 day ovariectomized (Ovx) rats. We then explored the regulation of PTHrP expression in these tissues 1,2,4,8 and 24 h following treatment with 20 μg/ kg 17 β-estradiol (E). In this study, efficacy of E-treatment was confirmed by measuring the temporal changes in PTHrP mRNA expression in the uterus. In pituitaries of sham-operated rats (~375 gm b.w.), PTHrP mRNA was easily detected and on day 28 post-Ovx we observed a 2-3 fold decrease in steady state levels of PTHrP mRNA in this tissue. Treatment with E caused a rapid and transient (appears at 1 h and returns back to basal levels by 8h) increase in PTHrP mRNA levels. Ovx also resulted in a decrease in steady state levels of PTHrP mRNA in the hypothalamus, and as seen in the pituitary, E-treatment stimulated a rapid, yet, transient increase in the accumulation of PTHrP mRNA in this tissue. Interestingly we found PTHrP mRNA to be abundant in the rat sciatic nerve. Localization of immunoreactive PTHrP to both axon lining cells and local arterioles suggests a role for this protein in peripheral nerve function. In contrast to what is seen in the pituitary and hypothalamus, Ovx did not alter the steady state levels of PTHrP in the sciatic nerve. Furthermore, levels of PTHrP mRNA in the sciatic nerve were unchanged in rats treated with E over the time course used in this study. These findings indicate that estrogen-responsiveness of PTHrP gene expression in the nervous system is tissue-specific. These data further extend our appreciation of the the complex nature of the physiological role for PTHrP in nervous system function.

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COEXPRESSION OF PTHrP AND PTH / PTHrP RECEPTOR mRNA IN VASCULATURE SUPPORTS A LOCAL MECHANISM OF ACTION IN CARDIOVASCULAR TISSUES.

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Although N-terminal fragments of PTHrP and PTH share classical calciotropic properties via their interactions with a common receptor in kidney and bone, these peptides also produce profound effects on the cardiovascular system including altering heart function and peripheral blood flow. Specific binding of PTH and PTHrP to vascular smooth muscle membranes, together with findings that PTHrP is a product of vascular tissues of both developmental and adult origin supports a possible local role for PTHrP in vascular function. To begin to address this possiblity, we examined the expression of mRNA encoding both PTHrP and the PTH / PTHrP receptor in the heart , aorta and several peripheral vascular tissues of male and female adult rats. In RNA blot analysis, a single 1.5 kb PTHrP mRNA was easily detected in total RNA prepared from heart, aorta, as well as pooled samples of isolated inguinal, femoral and tibial artery and vein. In the vascular tissues used in this study, we found relative levels of PTHrP mRNA to increase with distance from the heart. The variability in the levels of PTHrP mRNA in these tissues (tibial vessels > femoral ≥ inguinal > aorta≥ heart) suggests that the expression of PTHrP in the circulation is spatially regulated. Remarkably, we also found all of these tissues to express a single ~2.5 kb mRNA encoding the rat PTH / PTHrP receptor. However, in contrast to the variability in the levels of PTHrP mRNA seen in these tissues, the relative levels of PTH / PTHrP receptor mRNA were more constant throughout the cardiovascular tissues used in this study. Similar findings were observed in tissues obtained from either adult male or female adult rats. These data demonstrate that the gene for PTHrP and its receptor are coexpressed in tissues of the cardiovascular system and that the extent of this ligand / receptor interaction may, in part be controlled by levels of PTHrP synthesis which are known to be both spatially and temporally regulated. These findings further extend our understanding of how PTH / PTHrP may alter cardiovascular function via local ligand / receptor interactions.

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OCTREOTIDE, A SOMATOSTATIN ANALOG, SIMULTANEOUSLY DIMINISHED BONE RESORPTION AND ENHANCED FORMATION, IN A PATIENT WITH SEVERE PAGET'S DISEASE.

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We have recently reported (Bone & Mineral 17, S1:129, 1992), that octreotide (Oc) markedly reduces urinary excretion of calcium and partially lowers fecal Ca, resulting in a significant enhancement of positive Ca balance in children. Since bone contains ~ 99% of body Ca and since somatostatin or its analogs bind to bone cells in vitro and in vivo (Silve et al 1981, Bruns et al 1990), we proposed that Oc may have an anabolic effect on bone metabolism. To test this hypothesis, we studied the effect of Oc in a patient with very high bone turnover rate due to severe Paget's disease (alkaline phosphatase ~ 2,200 U/L, after 2 courses of pamidronate, the last given one year before the present study). Octreotide, 25 μg subcutaneously every 8h on day 1 followed by 3 days of Oc 50 μg q 8h caused no changes in serum Ca, P, and total serum alkaline phosphatase. However, serum osteocalcin rose from 41.0 ng/ml (day 0) to 75.1 (end of day 3; normal 2-7). Simultaneously the 24 h urinary excretion of deoxypyridinoline fell from 120.3 pmol/μmol creatinine to 46.1 (normal 4-21) (Tests performed by Nichols Institute). Since osteocalcin and deoxypyrinoline are markers of bone formation and resorption, respectively, this preliminary observation, in conjunction with the balance study mentioned above, suggests that Oc may stimulate bone anabolism.

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ACTIVATION OF THE IGF-II/CATION-INDEPENDENT MANNOSE 6-PHOSPHATE RECEPTOR BY ACID PHOSPHATASES STIMULATES A RAPID NET INFLUX OF Ca^{2+} IN BALB/C 3T3 FIBROBLASTS.

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Work in the early 1980's revealed a 250 kD membrane glycoprotein that bound insulin-like growth factor type II (IGF-II) with high affinity. Parallel work on what was thought to be a different receptor lead to the discovery and purification of a protein that specifically bound mannose-6 phosphate (M6P) containing peptides. The cation independent form of this mannose-6 phosphate receptor (CI-MPR) has recently been shown to have a common identity with the IGF-II receptor and so has been named, more appropriately, the IGF-II/CI-MPR. It is now accepted that the receptor has 15 accessible extracytoplasmic domains which include 1 site for binding IGF-II and 2 sites for binding M6P-containing peptides.

Recently, our laboratory has shown that uteroferrin, a tartrate resistant acid phosphatase (TRAP), and a human prostatic acid phosphatase (PAP), both of which contain M6P, have biologic effects similar to IGF-II in bone cells isolated from rat calvariae. Since both IGF-II and M6P containing peptides bind to the same receptor, it would not be surprising if both classes of peptides initiate biochemical effects through the same signal transduction pathway. Based on that premise, this study was undertaken in BALB/c 3T3 fibroblasts to determine if IGF-II as well as TRAP, PAP and M6P similarly stimulate DNA synthesis and Ca^{2+} influx. A net inward movement of Ca^{2+} would support the hypothesis that regardless of the ligand present, if each distinct ligand binds to the same receptor, initiation of the same signal transduction pathway must occur.

When quiescent BALB/c 3T3 cells were made competent by exposure to 10 ng/ml of PDGF for three hours and then primed with 10 nM EGF for 20 minutes, it was shown that IGF-II, TRAP,PAP and M6P all stimulated a net accumulation of Ca^{2+} ion (from 242% to 474% over control) in a dose dependent fashion. The net Ca^{2+} uptake was quantitated by measuring $^{45}Ca^{2+}$ influx and efflux from cells. This effect correlated with the ability of these molecules to enhance the proliferative rate of the cells (from 28% to 430% over control). These increases in DNA synthesis were determined by measuring changes in incorporation of [^{3}H]-thymidine in response to the various effectors. Control molecules such as non-M6P containing phosphatases, mannose and mannose-1 phosphate had no effect on Ca^{2+} influx or proliferation.

Preliminary results with the Ca^{2+} sensitive indicator fura-2 have supported the $^{45}Ca^{2+}$ flux data. Intracellular Ca^{2+} transients have been visualized in single BALB/c 3T3 fibroblasts with a fluorescence microscope in response to IGF-II, PAP and M6P. While further work needs to be done using this technique, it can be pointed out now that at least these three molecules are mobilizing intracellular Ca^{2+}, indicating the stimulation of the inositol phosphate signal transduction cascade. These data support our hypothesis that activation of the IGF-II/CI-MPR by either IGF-II or M6P-containing proteins utilizes the same second messenger signaling mechanism to evoke biological effects.